CRUK Cambridge Centre Clinical PhD project

PhD Project Title	**Targeting PPP1R15 in mesothelioma**
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Project Outline	**Aims and objectives** Nutrient deprivation and deregulated protein synthesis render cancer cells vulnerable to protein misfolding. Endoplasmic reticulum (ER) stress results from protein misfolding in the secretory pathway and has a profound effect on cancer progression (Clarke et al., 2014). A critical response to ER stress is mediated by the kinase PERK, but this is antagonised by the phosphatase PPP1R15A (Marciniak et al., 2004). PERK phosphorylates eIF2α to inhibit new protein synthesis and induce genes that allow the cell to respond to ER stress. In contrast, PPP1R15A selectively dephosphorylates eIF2α to oppose these effects of PERK. Recently, we discovered that the activity of PPP1R15A is regulated by its interaction with free G-actin (Chambers et al. 2015). The requirement of solid tumours for PERK signalling is now clear, but the impact of altered eIF2α phosphatase activity remains uncertain. However, we showed in malignant mesothelioma that loss of PPP1R15A protein expression correlates with tumour de-differentiation and worsening prognosis (Dalton et al, 2013). We have also observed loss of PPP1R15A from pre-malignant lesions in lung epithelia (unpublished). This loss of PPP1R15A in tumorigenesis does not reflect disruption of the *PPP1R15A* locus suggesting a post-transcriptional mechanism. We hypothesise that the mechanisms that regulate PPP1R15A turnover and activity will be useful targets in the development of novel therapies against mesothelioma and lung cancer. The purpose of this project is to elucidate these mechanisms and thus identify targets for drug development. **PPP1R15A degradation** - PP1R15A is an unstable protein with a half-life of <2 hours. Its N-terminal 60 residues are necessary for this instability and are sufficient to destabilise fusion proteins, but the ubiquitin ligases involved remain to be identified. Our unpublished results using SILAC mass spectrometry identify UBR4, a non-canonical N-recognin ubiquitin E3 ligase, to co-purify with PPP1R15A during inhibition of the proteasome. Importantly, we previously identified POE (*Drosophila* UBR4) to be the sole E3 ligase that co-purifies with *Drosophila* PPP1R15 (Chambers et al. 2015). One strand of this project will be to determine if UBR4 plays a major role in PPP1R15A degradation. In addition, non-biased techniques, e.g. CRISPR-based screens, will be used to identify further components of the PPP1R15A degradation machinery. **PPP1R15A regulation by the cytoskeleton** - Understanding the physiological role of the PPP1R15-actin interaction that we discovered (Chambers et al. 2015) may uncover strategies to modulate eIF2α phosphorylation that could be exploited for the treatment of cancer. Using a luciferase reporter cell lines, we can monitor the levels of eIF2α phosphorylation in real-time both in populations of cells and in individual cells. We will determine the physiological conditions in which G-actin availability affects PPP1R15 activity. The pathways involved in these physiological processes will then be targeted genetically and pharmacologically to ascertain their therapeutic

	potential.
Experimental plan	**PPP1R15A degradation** Using *Ubr4*$^{-/-}$ cells, the role of UBR4 in PPP1R15A stability will be determined. Our preliminary data suggest that UBR4 is not solely responsible for all PPP1R15A turnover and so forward genetics screens will be used to identify additional components of the PPP1R15A degradation machinery. We will determine if expression of these components predicts survival in mesothelioma by using the MesobanK 1000 Patient Tissue Microarray and its linked clinical data. As well as suggesting potential targets for intervention, these results may provide novel biomarkers of disease progression. **PPP1R15A regulation** Cells will be cultured in varying concentrations of growth factors to determine the effects of signal strength on the formation of an active PPP1R15A-actin complex and on its phosphatase activity. Reporter-based imaging techniques will be used to provide additional sensitivity and temporal resolution. Epithelial to mesenchyme transition (EMT) contributes to cancer metastasis by increasing cell motility and invasiveness, in part through reorganisation of the actin cytoskeleton. We will compare motile and immotile cells for differences in PPP1R15A-actin complex formation, eIF2α phosphatase activity and turnover. We will then manipulate pathways driving EMT in malignancy and test for alterations of PPP1R15 activity.
Main Techniques	• Western blotting • CRISPR/Cas9 gene editing • Luciferase reporter assays • Mesothelioma tissue culture
Key References	**1.Chambers, J.E., Dalton, L.E., Clarke, H.J., Malzer, E., Dominicus, C.S., Patel, V., Moorhead, G., Ron, D., and Marciniak, S.J.** *2015. Actin dynamics tune the integrated stress response by regulating eukaryotic initiation factor 2alpha dephosphorylation. eLife 4, doi: 10.7554/eLife.04872*. **2.Clarke, H.J., Chambers, J.E., Liniker, E., and Marciniak, S.J.** *2014 Endoplasmic reticulum stress in malignancy. Cancer Cell 25, 563-573.* **3.Dalton LE, Clarke HJ, Knight J, Lawson MH, Wason J, Lomas DA, Howat WJ, Rintoul RC, Rassl DM & Marciniak S.J.** *2013 The endoplasmic reticulum stress marker CHOP predicts survival in malignant mesothelioma. Br J Cancer 108: 1340-7.* **4. Marciniak, S.J., Yun, C. Y., Oyadomari, S., Novoa, I., Zhang, Y., Jungreis, R., Nagata, K., Harding, H. P., and Ron, D.** *2004. CHOP induces death by promoting protein synthesis and oxidation in the stressed endoplasmic reticulum. Genes Dev 18, 3066-3077.*